the notion that phenotypic heterogeneity of endothelial cells within blood vessels is critical to proper development and function. The work provides an example of how changes to cell shape rather than cell numbers dramatically affects blood vessel phenotype and tumor progression. Finally, blood vessel normalization affects tumor invasion and metastasis, suggesting that endothelial oxygen sensing may represent a new target of cancer therapy.

## REFERENCES

Bergers, G., and Hanahan, D. (2008). Nat. Rev. Cancer *8*, 592–603.

Dutta, D., Ray, S., Vivian, J.L., and Paul, S. (2008). J. Biol. Chem. *283*, 25404–25413.

Hanahan, D., and Folkman, J. (1996). Cell *86*, 353–364.

Jain, R.K. (2005). Science *307*, 58–62.

Kendall, R.L., and Thomas, K.A. (1993). Proc. Natl. Acad. Sci. USA *90*, 10705–10709.

Le Bras, A., Lionneton, F., Mattot, V., Lelievre, E., Caetano, B., Spruyt, N., and Soncin, F. (2007). Oncogene *26*, 7480–7489.

Mazzone, M., Dettori, D., Leite de Olivera, R., Loges, S., Schmidt, T., Jonckx, B., Tian, Y.-M., Lanahan, A.A., Pollard, P., Ruiz de Almodovar, C., et al. (2009). Cell, this issue.

Semenza, G.L. (2003). Nat. Rev. Cancer *3*, 721–732.

Taddei, A., Giampietro, C., Conti, A., Orsenigo, F., Breviario, F., Pirazzoli, V., Potente, M., Daly, C., Dimmeler, S., and Dejana, E. (2008). Nat. Cell Biol. *10*, 923–934.

Takeda, K., Ho, V., Takeda, H., Duan, L.-J., Nagy, A., and Fong, G.-H. (2006). Mol. Cell. Biol. *26*, 8336–8346.

# In DNA Replication, the Early Bird Catches the Worm

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**The initiation of DNA replication is a complex, multistep process with important implications for genomic stability. In this issue, Wu and Nurse (2009) find that initiation factors are differentially recruited to replication origins. They uncover evidence suggesting that the efficiency of this recruitment may determine whether and when an origin is used to initiate DNA replication in S phase.**

Most cells spend a large portion of the cell cycle preparing to replicate their DNA. Replication initiates from regions in the chromosomal DNA called replication origins in a process referred to as origin firing. These origins attract the six origin recognition complex (ORC) proteins during mitosis. The binding of ORC proteins to the origin serves to initiate a multistaged process involving the recruitment of a series of different proteins that results in the separation of the two DNA strands, loading of the DNA polymerase, and initiation of DNA replication (Figure 1). Yet, virtually nothing is known about the mechanisms by which these regions, crucial to replication, are selected and when this selection is made. Furthermore, unlike in prokaryotes, in eukaryotes there are no known characteristics that can help to predict whether a given DNA sequence can serve as an origin of DNA replication. A single mammalian cell harbors tens of thousands of potential replication origins, yet only a subset of these is used in each particular S phase. Thus, the activities of these origins must be strictly regulated. Indeed, defective control of DNA replication can lead to mutations, genomic instability, and cancer. In this issue of *Cell*, Wu and Nurse (2009) find evidence suggesting that in the fission yeast, *Schizosaccharomyces pombe*, the ability of an origin to attract ORC proteins during mitosis determines to a large extent the timing of initiation factor assembly at the origin and whether that origin is selected as a replication initiation site in the following S phase.

To examine how replication origins are selected, Wu and Nurse measured the kinetics with which initiation factors, including ORC proteins, bind to origins in fission yeast. The authors found that origins known to be efficient ("good" origins used in almost every S phase) bind to ORC proteins earlier and with greater affinity than origins that are not frequently used ("poor" origins). This correlation could also be seen in the efficiency with which prereplicative complexes (pre-RCs) and pre-initiation complexes (pre-ICs) are formed at different origins. Furthermore, if the time available for ORC proteins to bind at origins is increased by adding a drug that prolongs mitosis, Wu and Nurse observed that efficient origins become less efficient, whereas poor origins are used more frequently. These experiments substantiate the claim that the pattern of ORC protein binding determines which potential origins are actually used in the ensuing S phase. It is possible that increasing the length of mitosis allows ORC protein binding to become equalized between

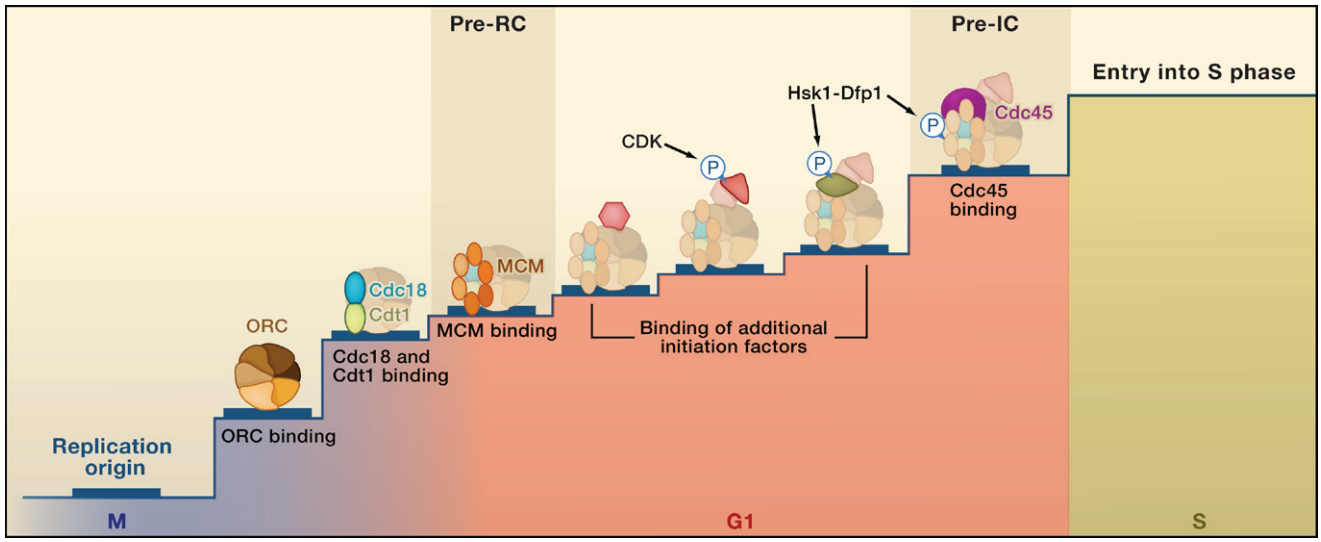


**Figure 1. Preparing a Fission Yeast Replication Origin for DNA Replication**

The steps toward preparing a replication origin for initiation of DNA replication are shown. The six origin recognition complex (ORC) proteins first bind to the replication origin during mitosis. This allows the binding of Cdc18 (a homolog of mammalian CDC6) and Cdt1. These proteins recruit the six Mcm proteins that constitute a putative DNA helicase for unwinding the two strands of DNA, thus forming the pre-replicative complex (pre-RC). Several intervening steps then occur where additional initiation factors bind at the origin. Phosphorylation (arrow) of some of these factors by cyclin-dependent kinase (CDK) or by Hsk1 (homolog of Cdc-seven kinase 1) and its regulatory subunit Dfp1 (DBF-four in *pombe* 1) is thought to be required for loading of Cdc45 onto the origin. Loading of the protein Cdc45 at the origin completes formation of the pre-initiation complex (pre-IC). The pre-IC prepares the origin for the loading of primase and DNA polymerase, enzymes that are essential to DNA replication in S phase. Hsk1-Dfp1 kinase activity (arrow) is thought to be required for activation of the Mcm helicase. (Steeper steps represent the loading of factors known to be subject to regulation. Proteins already present at the origin at each step are shown as fainter images).

the different origins such that efficient origins do not bind as many ORC proteins and poor origins are able to bind more. However, it is more likely that a longer mitosis allows poor origins to accumulate sufficient amounts of ORC proteins to enable them to compete with other origins for additional initiation factors such as Cdc18, Cdt1, and Mcm proteins (Figure 1). The reduced efficiency in the firing of good origins observed during an extended mitosis may also be explained by competition between origins such that when a poor origin eventually fires, it may prevent the firing of a neighboring efficient origin (this origin is passively replicated instead).

The binding of ORC proteins is crucial for the timing of origin firing, but several other factors also have to be loaded onto the DNA at the origin for replication initiation to occur (Figure 1). Many of these steps are likely to be regulated, both during a normal cell cycle and during the activation of cell cycle checkpoints. It is known that many more pre-RCs are formed at origins than undergo firing during the subsequent S phase. Therefore, factors that act downstream of ORC protein binding at the origin clearly also limit origin usage. In an attempt to identify these limiting events, Wu and Nurse induced overexpression in fission yeast cells of the initiation factors Cdc45, Hsk1 (homolog of Cdc-seven kinase 1), or Dfp1 (DBF-four in *pombe* 1), which all act late in the initiation program that leads to DNA replication (Figure 1). The Hsk1 kinase and its regulatory subunit Dfp1 are required at the origin for the loading of Cdc45 and for the activation of the Mcm helicase (Figure 1). Cdc45 is thought to act as a cofactor for the Mcm helicase. The authors observed an increase in origin efficiency across the genome when any of the three factors is overproduced. Although this suggests that each of the proteins are limiting in some way during the preparation for replication initiation, it does not seem likely that the actions of all three proteins normally limit origin usage. Instead, it may be that increased amounts of any individual factor can compensate for the activity of other limiting initiation factors and advance the initiation process, thereby facilitating origin use during S phase.

The amount of the Hsk1-Dfp1 kinase in a fission yeast cell has been previously shown to influence the selection of origins for replication initiation (Patel et al., 2008). The same study further found that stimulated or depressed origin usage as the result of altered Hsk1-Dfp1 levels caused genomic instability, highlighting the importance of activating the right set or the right number of origins. Further support for a regulatory role of this kinase comes from experiments in budding yeast, where it has been observed that premature initiation of DNA replication can be triggered in the presence of elevated levels of the Dfp1 homolog DBF4 if target proteins of the cyclin-dependent kinase (CDK) were mutated to mimic their phosphorylated states (Zegerman and Diffley, 2007). As the kinase activities of both Hsk1-Dfp1 and CDK also promote Cdc45 loading (Figure 1), these data further argue that the loading of Cdc45 is a limiting and regulated step during replication initiation. Two other initiation factors, Cdc18 and

Ctd1, have also been suggested to limit origin firing. Previous studies in fission yeast have shown that firing can occur multiple times at the same origins in cells overproducing either Cdc18 or both Cdc18 and Cdt1 (Nishitani and Nurse, 1995; Nishitani et al., 2000), resulting in overreplication. It is not yet clear whether the overproduction of Cdc45, Hsk1, or Dfp1 results in the same type of overreplication or whether it merely changes the pattern of origin usage.

The work by Wu and Nurse gives us a handle on the sequence of regulatory events that lead up to DNA replication. In light of this new information regarding the potential regulatory roles played by ORC protein binding in determining origin usage, future work should undoubtedly be focused on identifying the signals that initiate ORC loading in both cycling cells and cells returning from quiescence, thereby setting the stage for a well-regulated S phase.

## REFERENCES


Nishitani, H., Lygerou, Z., Nishimoto, T., and Nurse, P. (2000). Nature *404*, 625–628.

Nishitani, H., and Nurse, P. (1995). Cell *83*, 397–405.

Patel, P.K., Kommajosyula, N., Rosebrock, A., Bensimon, A., Leatherwood, J., Bechhoefer, J., and Rhind, N. (2008). Mol. Biol. Cell *19*, 5550–5558.

Wu, P.-Y.J., and Nurse, P. (2009). Cell, this issue.

Zegerman, P., and Diffley, J.F. (2007). Nature *445*, 281–285.


# A STIMulus Package Puts Orai Calcium Channels to Work


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**Activation of plasma membrane calcium channels by depletion of endoplasmic reticulum (ER) calcium stores is important for calcium entry in many cell types. Park et al. (2009) now show that direct binding of the ER protein STIM to tetramers of the Orai1 calcium channel in the plasma membrane triggers opening of this channel.**


Calcium ions ($Ca^{2+}$) are ubiquitous intracellular signals that trigger hundreds of biological processes. In addition to $Ca^{2+}$-permeant channels in the plasma membrane that allow for rapid increases in the level of cytoplasmic $Ca^{2+}$, cells also possess an intracellular $Ca^{2+}$ distribution system. In many types of cells, $Ca^{2+}$ depletion from the endoplasmic reticulum (ER) leads to the rapid activation of plasma membrane $Ca^{2+}$ channels in a process known as store-operated $Ca^{2+}$ entry. Park et al. (2009) now examine the mechanistic basis of store-operated $Ca^{2+}$ entry and provide evidence that it is stimulated by the direct binding of an ER protein, STIM1, to the plasma membrane calcium channel Orai1.

The ER is an ideal $Ca^{2+}$ capacitor by virtue of its large surface area and storage capacity. The capacitor is charged by the smooth ER $Ca^{2+}$ ATPase (SERCA) pump and discharged via $Ca^{2+}$-permeant channels, such as inositol triphosphate receptors ($IP_3R$) and ryanodine receptors (RyR). G protein and tyrosine kinase receptor-activated phospholipase C (PLC) hydrolyzes plasma membrane-specific phosphatidylinositol bisphosphate ($PIP_2$) to release soluble $IP_3$. Within seconds, $IP_3$ gates the ER $IP_3R$ channel to increase cytoplasmic $Ca^{2+}$. In reciprocal signaling over the course of minutes, $Ca^{2+}$ entry at the plasma membrane is activated via a message from the $Ca^{2+}$-depleted ER. This mechanism, called store-operated $Ca^{2+}$ entry, is mediated by slow, tiny, and highly selective $Ca^{2+}$ channels known as CRAC ($Ca^{2+}$ release-activated $Ca^{2+}$) channels that are activated when the $Ca^{2+}$ concentration in the ER falls. Most importantly, a declining ER $Ca^{2+}$ concentration, but not an increasing cytoplasmic $Ca^{2+}$ concentration, activates CRAC channels. This is a crucial distinction that separates CRAC channels from periplasma membrane $Ca^{2+}$-activated transient receptor potential (TRP) channels and $K^+$ channels.

The sensor and reciprocal messenger from the ER to the plasma membrane in store-operated $Ca^{2+}$ entry were sought for over 15 years (Parekh and Putney, 2005). The crucial break came when STIM, a single transmembrane-spanning domain protein primarily residing in the ER, was found to be essential for activation of store-operated $Ca^{2+}$ entry in *Drosophila* (Stim) and mammalian (STIM1) cells (Roos et al., 2005). STIM can be likened to a buoy, with its N terminus inside the ER and its C-terminal protein-interaction domains bobbing in the cytoplasm (Figure 1). When the ER $Ca^{2+}$ concentration falls, STIM proteins accumulate within minutes